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Ultrastructure of *Frenkelia microti* in Prairie Voles Inoculated with Sporocysts from Red-tailed Hawks

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ABSTRACT: The ultrastructure of *Frenkelia microti* tissue cysts was examined in experimentally inoculated prairie voles (*Microtus ochrogaster*). Tissue cysts were lobate, thin-walled, and divided into compartments by septa. The host cell nucleus was often hypertrophic. Tissue cysts were enclosed by a primary cyst wall composed of the parasitophorous vacuole membrane that was highly ornamented with 0.2-μm, electron-dense, knoblike projections. The primary cyst wall was supported by granular ground substance. The entire tissue cyst wall was about 0.7 μm in thickness. Ground substance formed septa that extended into the cyst to produce compartments. Osmiophilic bodies about 60 nm in diameter and associated with microfilaments were observed in the ground substance and septa. Membrane fragments and membranous bodies were often observed within compartments. Metrocytes and mature bradyzoites were present in compartments both centrally and at the periphery of tissue cysts. Metrocytes divided by endodyogeny to produce bradyzoites. Rhoptries and micronemes were present in the anterior ¼ to ⅓ of each bradyzoite. The bradyzoite nucleus was often elongate and located in the posterior ½ of the parasite. Amylopectin granules were most abundant in the posterior portion of the bradyzoite.

KEY WORDS: ultrastructure, tissue cyst, prairie vole, *Microtus ochrogaster,* red-tailed hawk, *Buteo jamaicensis,* Apicomplexa, Sarcocystidae, *Frenkelia microti.*

Frenkelia microti (Findlay and Middleton, 1934) Biocca, 1968, is an obligatory, heteroxenous coccidium that produces tissue cysts in the brains of a variety of rodents (Dubey et al., 1989). Recently, Upton and McKown (1992) reported that sporocysts isolated from the feces of a red-tailed hawk (*Buteo jamaicensis*) collected in Kansas, U.S.A., induced infections in orally inoculated prairie voles (*Microtus ochrogaster*). The tissue cysts, found only in the brain, resembled *F. microti* when examined with light microscopy. They were unable to demonstrate infection in white-footed mice (*Peromyscus leucopus*) orally inoculated with sporocysts of the same isolate.

Previous studies of the ultrastructure of tissue cysts of *F. microti* have been done on naturally infected hosts, often using poorly fixed material (Tadros et al., 1972; Hayden et al., 1976; Kennedy and Frelier, 1986). The present study was conducted to examine the ultrastructure of the tissue cysts of *F. microti* in an experimentally infected prairie vole.

Materials and Methods

Tissue cysts were obtained from the brain of a prairie vole inoculated orally 114 days previously with *Frenkelia*-like sporocysts obtained from a red-tailed hawk (SAR-13 of Upton and McKown, 1992). Small portions (about 2–3 mm^3) of brain were fixed in 2.5% (v/v) glutaraldehyde in phosphate-buffered saline, post-fixed in 1% osmium tetroxide, dehydrated in ethanols, and embedded in Spurr's plastic (Polysciences, Inc., Warrington, Pennsylvania). Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 transmission electron microscope operating at 60 kV. One-micrometer thick sections were stained with toluidine blue and examined with light microscopy. Additional portions of the brain were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, sectioned at 8 μm and stained with hematoxylin and eosin (H&E) or periodic acid-Schiff (PAS) for light microscopic examination.

Results

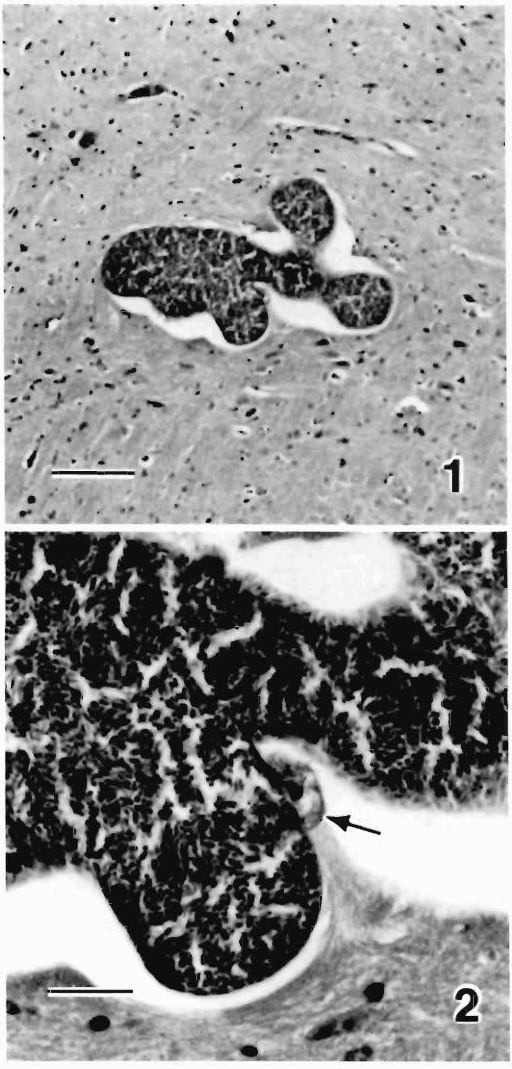
Seven tissue cysts were examined with light microscopy; 6 were lobate and irregular in shape (Fig. 1) and 1 was spherical. The tissue cyst walls were smooth and less than 0.5 μm thick. Septa were present but were difficult to visualize. Metrocytes contained few PAS-positive granules, whereas bradyzoites were intensely PAS-positive. Metrocytes were difficult to visualize in H&E stained sections but were readily seen in toluidine blue stained sections where they were faint blue in contrast to the dark blue bradyzoites. A hypertrophic nucleus of the infected host cell (Fig.

2) was seen adjacent to 2 tissue cysts. No other lesions were observed in sections of brain examined with light microscopy.

Three tissue cysts were examined with transmission electron microscopy (TEM). Their structure was similar (Figs. 3–5). Each tissue cyst was enclosed by a primary cyst wall composed of the parasitophorous vacuole (PV) membrane which was highly ornamented with electron dense knoblike projections. The knoblike projections were about 0.2 μm in length and were composed only of the PV membrane at the base. Electronlucent areas resembling holes were observed in portions of the primary cyst wall. They apparently resulted from sectioning through the bases of the knoblike projections (Fig. 5). The primary cyst wall was supported by granular ground substance, which composed the remainder of the tissue cyst wall. The entire tissue cyst wall was about 0.7 μm. The ground substance extended into the tissue cyst in the form of septa to produce a network of ill-defined compartments. Osmiophilic bodies about 60 nm and associated with microfilaments were often present in the ground substance and septa (Fig. 4). Metrocytes and mature bradyzoites were in compartments both centrally and at the periphery of tissue cysts (Fig. 3). However, some compartments at the periphery of tissue cysts appeared to contain only metrocytes. Membrane fragments and membranous bodies were often present within compartments (Fig. 4).

Metrocytes divided by endodyogeny to produce bradyzoites (Figs. 3, 4). Most major organelles disappeared prior to the beginning of bradyzoite production, but the conoid, micronemes, amylopectin granules, and mitochondria persisted well into bradyzoite formation. Bradyzoite anlagen developed in association with the parent nucleus, which became horseshoe-shaped and migrated into each of the developing bradyzoites. The conoids, rhoptries, micronemes, and subpellicular microtubules reappeared as the developing bradyzoites increased in size. The inner membrane complex of the metrocyte degenerated, and eventually the outer membrane of the developing bradyzoites fused with metrocyte outer pellicle membrane as mature bradyzoites were produced. Micropores were present in both metrocytes and bradyzoites.

Bradyzoites contained a conoid and at least 4 rhoptries. The ducts of rhoptries were often seen entering the conoid (Fig. 6). Numerous rodlike micronemes were present in the anterior ¼ to ⅓ of the bradyzoite (Figs. 6, 7). The bradyzoite nucleus was usually elongate and was located in the posterior ½ of the parasite (Fig. 7). Twenty-two subpellicular microtubules were present and they extended from the anterior end to about ½ the length of the bradyzoite. Amylopectin granules were present throughout the bradyzoites but were most abundant in the posterior portion of bradyzoites.



Figures 1, 2. Photomicrographs of *Frenkelia microti* tissue cysts in hematoxylin and eosin stained tissue sections. 1. Lobate tissue cyst. Note absence of host inflammatory response. Bar = 100 μm. 2. Higher magnification of tissue cyst in Figure 1. Note the hypertrophic host cell nucleus (arrow). Bar = 25 μm.

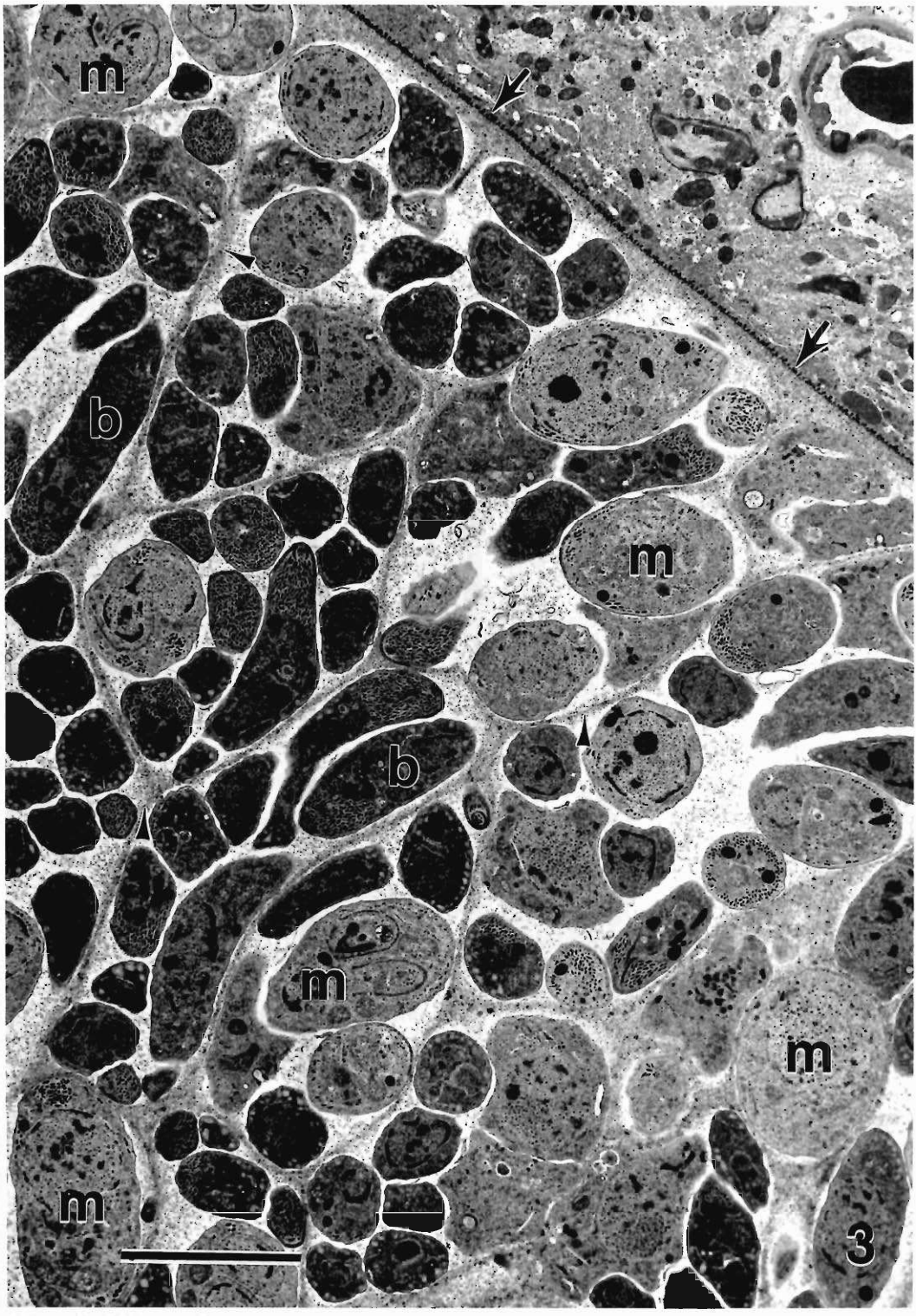


Figure 3. Electron micrograph of a portion of a *Frenkelia microti* tissue cyst. Numerous metrocytes (m) are located at the periphery and centrally within the tissue cyst. The compartments are separated by septa (arrowheads) and most contain both bradyzoites (b) and metrocytes. Arrows point to the tissue cyst wall. Bar = 5.0 μm.

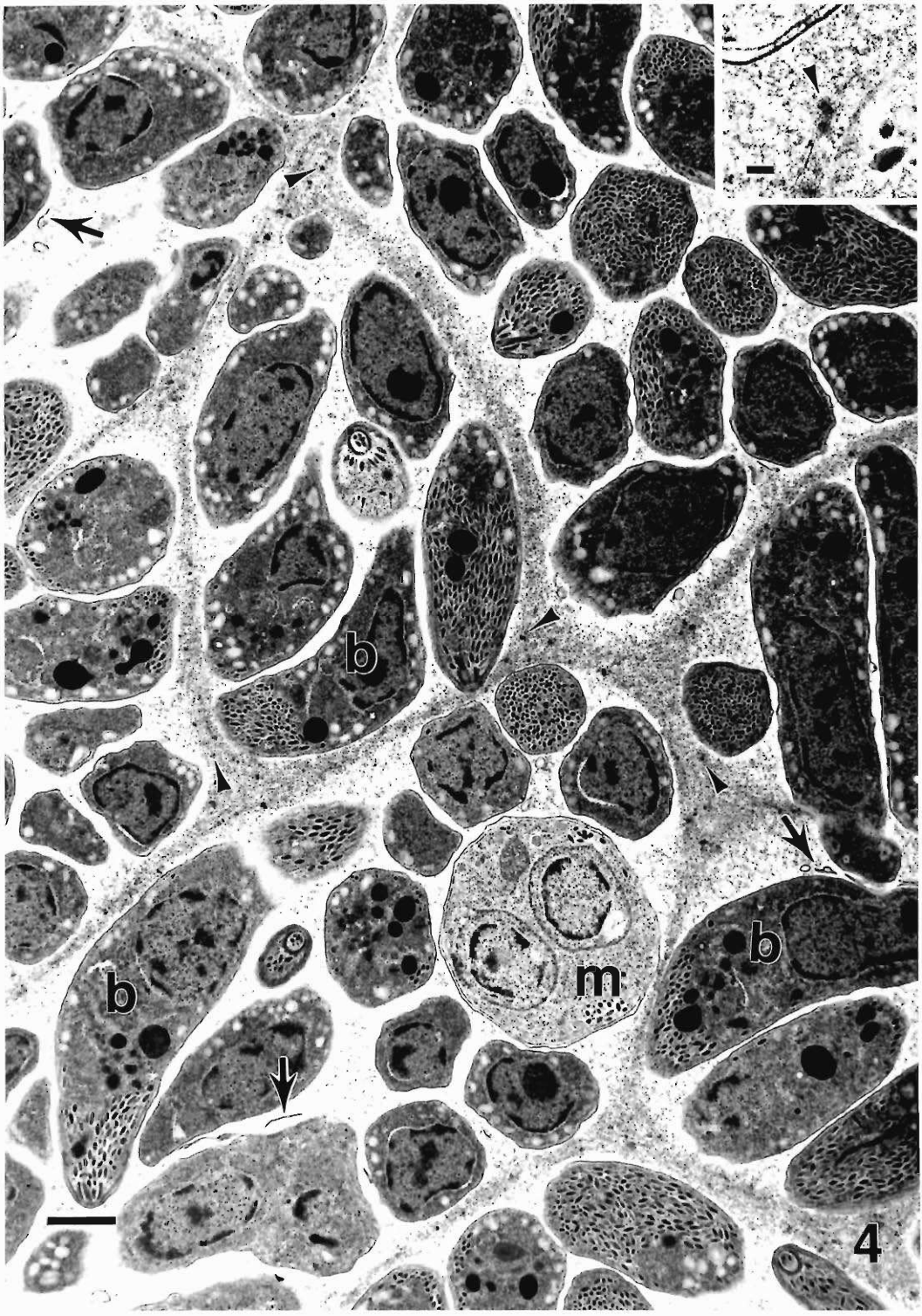


Figure 4. Electron micrograph of compartments within a *Frenkelia microti* tissue cyst. The compartments in this section contain mostly bradyzoites (b) and only an occasional metrocyte (m). Note that the labeled bradyzoites have micronemes that are confined to the anterior portion of the bradyzoite. Membrane fragments (arrows) are visible in several of the compartments, and osmiophilic bodies (arrowheads) are visible in septa. Bar = 1.0 μm. INSET. Osmiophilic body (arrowhead) demonstrating its association with microfilaments. Bar = 100 nm.

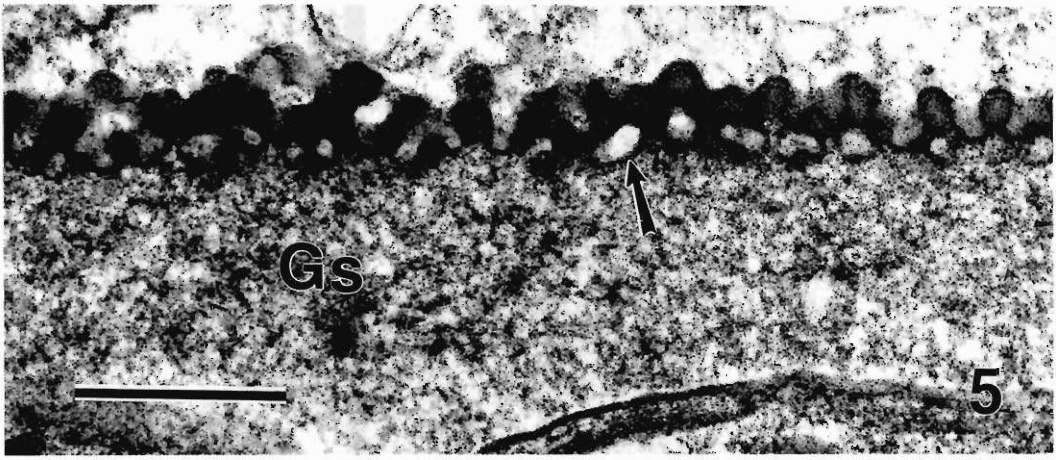


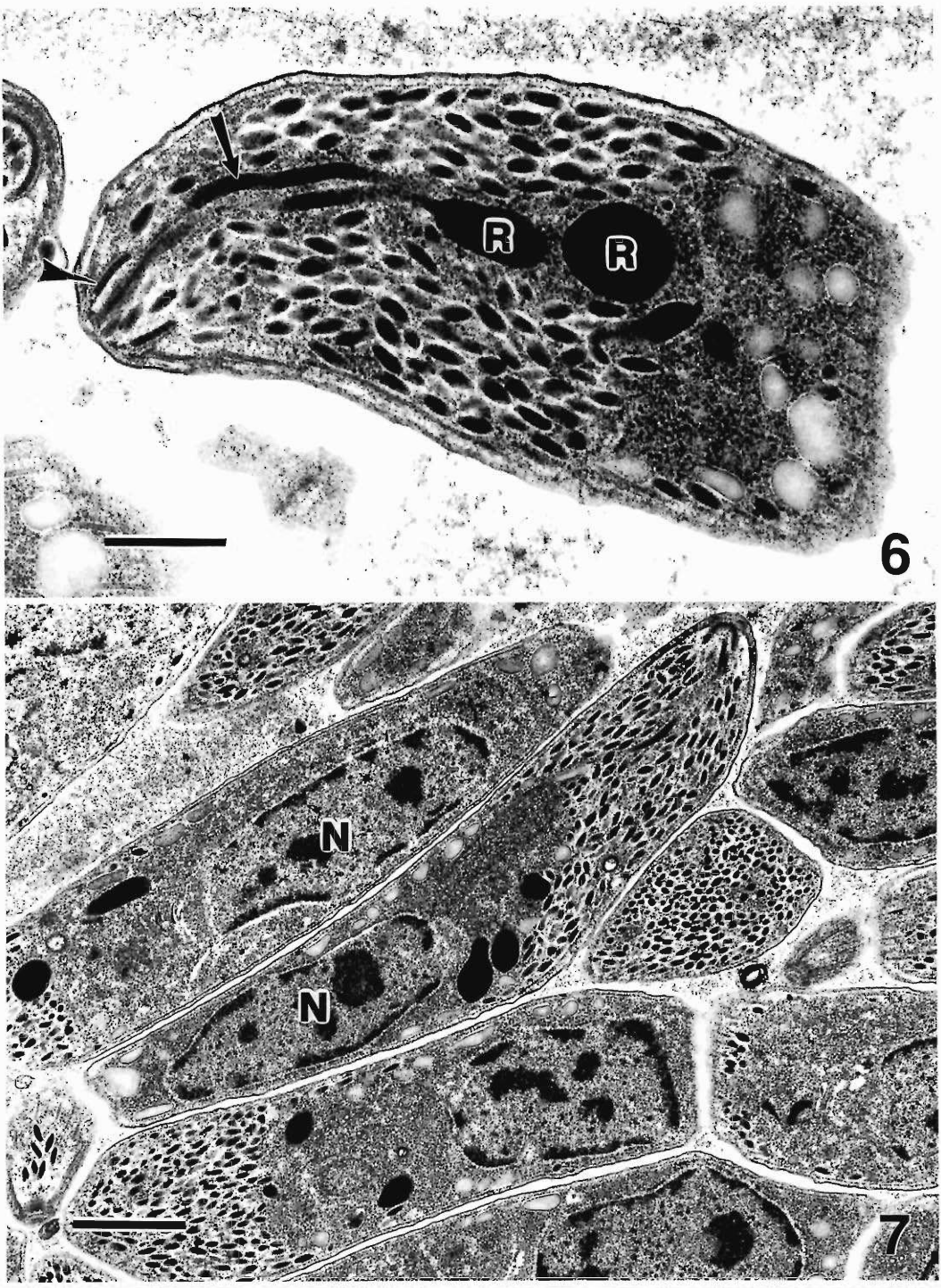
Figure 5. Tissue cyst wall of *Frenkelia microti*. Note the knoblike projections of the primary cyst wall and the apparent hole (arrow) that is a result of the plane of sectioning. The granular ground substance (Gs) is directly beneath the primary cyst wall. Bar = 0.5 μm.

Discussion

The light microscopic appearance of *Frenkelia microti* tissue cysts in brain sections of a prairie vole in this study was similar to tissue cyst structure reported by others in a variety of naturally infected mammals (Frenkel, 1956; Karstad, 1963; Hayden et al., 1976; Kennedy and Frelier, 1986). We did not observe lesions associated with tissue cysts, consistent with reports by others (Frenkel, 1956; Karstad, 1963). However, some reports indicate that microscopic lesions are associated with infection (Hayden et al., 1976; Kennedy and Frelier, 1986). The single spherical *F. microti* tissue cyst we observed may have been an artifact of tissue sectioning or an immature cyst. Geisel et al. (1979) reported that young *F. microti* tissue cysts were spherical.

Tadros et al. (1972) gave a brief description of the ultrastructure of tissue cysts of *F. microti* and *F. glareoli* in naturally infected voles (*Microtus agrestis* and *Clethrionomys glareolus,* respectively). Their report is difficult to interpret because tissues were poorly fixed and it is often unclear which species of *Frenkelia* they are describing. They did not provide an adequate description of the tissue cyst wall nor did they report the presence of osmiophilic bodies in septa or the membranous fragments and bodies seen within compartments. The origin of and significance of the osmiophilic bodies observed in the septa in the present study is unknown. The membranous fragments and bodies observed in compartments may represent byproducts of endodyogeny.

The structure of *F. microti* tissue cysts are similar to those of *Sarcocystis montanaensis* Dubey, 1983, found in heart, tongue, and various skeletal muscles of *Microtus* species (Dubey, 1983; Lindsay et al., 1991, 1992). Sarcocysts of some species of *Sarcocystis* can be found in the brain (Dubey et al., 1989), but sarcocysts of *S. montanaensis* were not reported in the brain tissue of the few voles that have been examined (Lindsay et al., 1992). The lobate structure of *F. microti* tissue cysts and their location in the brain can be used to differentiate it from *S. montanaensis* with light microscopy. Additionally, TEM can be used to demonstrate that bradyzoites of *F. microti* have micronemes which are restricted to the anterior region, whereas bradyzoites of *S. montanaensis* have micronemes that are located both anteriorly and posteriorly.



Figures 6, 7. Bradyzoites of *Frenkelia microti*. **1.** Anterior end of a bradyzoite demonstrating a conoid (arrowhead), rhoptries (R), and numerous rodlike micronemes. Note the duct of one rhoptry (arrow). Bar = 0.5 μm. **7.** A group of bradyzoites. Note the elongate nucleus (N) that is present and that the micronemes are located in the anterior portion of the bradyzoite. Bar = 1.0 μm.

Acknowledgments

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Zoological Nomenclature

The following Applications were published on 26 March 1992 in Vol. 49, Part 1 of the *Bulletin of Zoological Nomenclature.* Comment or advice on these Applications is invited for publication in the *Bulletin,* and should be sent to the Executive Secretary, I.C.Z.N., % The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

Case 2251 *Bucephalus* Baer, 1827 and *B. polymorphus* Baer, 1827 (Trematoda): proposed conservation in their accepted usage

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Abstract. The purpose of this application is to conserve in their accepted usage the generic and specific names of an important trematode parasite of freshwater fishes—*Bucephalus polymorphus* Baer, 1827. The name *B. polymorphus* was based on cercariae, but it has been shown that these develop into the adult trematode first named as *Rhipidocotyle campanula* (Dujardin, 1844), a senior synonym of *R. illensis* (Ziegler, 1883). A neotype for *B. polymorphus* is proposed to avoid transfer of this long recognized name to *R. campanula,* with resulting confusion at both generic and specific levels.